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METALS AND ORGANOCHLORINE COMPOUNDS IN LITHUANIAN HONEY

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The quality and value of honey, as natural bio-product, depends on its sort and origin. The quantitative and qualitative relations of chemical elements are characteristic to each blossom of the plant from each region of the country, so general quantity of mineral materials depends on the location. The amounts of heavy metals (HM) – Pb, Cd, Cu, Zn, Sr, Rb, Ba, rare elements – Ce, La, U and organic chlorine compounds – DDT, its metabolites: DDD and DDE also α-HCH and γ-HCH and polychlorinated biphenyls (PCB) – were determined in samples of honey collected from different parts of Lithuania.

It was established, that the concentration of heavy metals in Lithuanian honey varied in a large range: Pb 3.20÷24.10 μg/kg, Cd 3.90÷16.50 μg/kg, Cu 110.60÷389.40 μg/kg, and Zn 564.30÷5008.20 μg/kg. The determined concentrations of microelements stay within the Maximum Tolerable Limit (MTL) of Lithuanian Standards of Hygiene. In turn, 2248.50 μg/kg of Rb was found in honey, which was collected in the forest of Labanoras. According to the Rb amounts in honey, it can be assorted into honey of mead and forest. The concentrations of heavy metals determined in Lithuanian honey are lower than in honey of some other EU Countries.

Polychlorinated biphenyls were not found in the examined samples of Lithuanian honey and only the prints of organochlorine compounds were observed.

INTRODUCTION

The demand for bio-products has grown recently. Honey is natural, very nutritious bio-product with perfect properties: bactericidical, conservation, and organism stimulating ones. Its value depends on the sort, location, environment, collection time, and climatic conditions [Gaidamavicius, 2005]. In natural honey, there are up to 75% of carbohydrates (mainly fructose and glucose), organic acids (oxalic, lactic, malic, tartaric, and citric), amino acids (praline, phenylalanine), hydroxyamines, vitamins, ferments, mineral materials and microelements [Kubiliene *et al.*, 2004]. Lack, excess or misbalance of these microelements could cause health problems to human organisms. It has been observed that composition of mineral materials in honey is identical to human blood [Kubiliene *et al.*, 2004]. Microelements are very biologically active; they ensure the natural development of physiological reactions, take part in metabolism and impact general metabolism, germination, circulatory systems and influence the reproduction of organism as catalysts of various biochemical reactions. Microelements are the constitutive parts of the structures of different active bio-compounds: zinc, cooper, manganese – in ferments, cobalt – in vitamins, iodine, and cobalt – in hormone, cooper and iron – in the respiratory ferments. Apart from microelements that are indispensable for human organisms, there is a group of microelements that are harmful – lead, cadmium, mercury, aluminum [Matei *et al.*, 2004; Piekut *et al.*, 2004; Smalinskiene *et al.*, 2001; Terrab *et al.*, 2005; Waili *et al.*, 2003].

Organochlorine compounds are used as a chemical protector for plants. The most hazardous is 1,1,1-trichloro-2,2--di(4-chlorophenyl)ethane (DDT) and its metabolites, they are unexploited presently, however because of the stability and resistance to environmental influence, they circulate into biota away [Chauzat *et al.*, 2006; Tsimeli *et al.*, 2008]. The toxicity of polychlorinated biphenyls (PCB), hexachlorocyclohexanes (HCH), DDT and its metabolites depends on their concentration, durational impact, chlorination degree, isomeric composition of mixture. They could induce dermatoses, canker of the liver, inhibit immunity and protective reactions of organism, influence the nervous system and lungs, and trigger cancerous troubles. Consequently, it is very important to evaluate the pollution of every foodstuff, including the honey.

The concentration of microelements and pesticides that can cause toxic danger, including heavy metals in nutritional products, is regulated by establishing tolerable limits of their concentration. Maximum Tolerable Limits (MTL) of chemical elements are determined in accordance to recommendations of World Health Organization (WHO) and Food and Agriculture Organization of the United Nations state (FAO), nutritional act of the Republic of Lithuania as well as the real situation in the country. The general parameters of safety of nutrition products are regulated by the Lithuanian Standards of Hygiene HN 54:2001. According to this act, the levels of Pb and Cd are limited in all products and the concentrations of Cu, Zn, Sn (tin) and Hg – in some nutrition products. In addition, the quality of honey is regulated

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by the Lithuanian Standard LST ISO 1208:2003 and the act No.5 (2000-01-10) of the Ministry of Agriculture of the Republic of Lithuania. Health Institutions in many countries approve the recommended safety standards of the nutrition, where the basic amounts (biologically indispensable) of the intake of the microelements per day are indicated.

The aim of this work was to determine the concentration of heavy metals, excess of which could cause toxic danger – Pb, Cd, Cu, Zn, as well as other biologically required elements - Sr, Rb, Ba and rare elements - Ce, La, U and organochlorine compounds (COC) – DDT, DDD, DDE, HCH and PCB in Lithuanian honey; to evaluate the levels of contamination of Lithuanian honey with HM, as well as to compare the research results with data found by other investigators.

MATERIALS AND METHODS

Samples of honey collected in August of 2004 in different sampling points of Lithuania were analysed (Figure 1).

Gas chromatography technique was used for the determination of the levels of COC. The group of COC was extracted from the honey with a mixture of hexane and acetone. The extract was concentrated and products were purified by column chromatography using an eluent mixture of hexane, dichloromethane and petrol ether in a volume ratio of 8:1:1. The solvents were rotary evaporated and the products were dissolved in petrol ether. The gas chromatograph "Shimadzu–GC14A" was used for the analysis of the received samples.

Concentrations of metals were determined using double focusing and high resolution ICP–MS model "Element" (Finnigan MAT, Bremen) in the Federal Institute of Consumer Health Protection and Veterinary Medicine in Berlin, Germany. This method enables determination of the amounts of metals that are lower than 10^{-12} g/kg.

As the concentrations of metals may vary, so the honey samples of concentrations of 0.5% and 5% were prepared. 0.5 g and 5.0 g of honey were injected into two 100 mL volumetric flasks and 30-50 mL. of distilled water were poured over it. The honey was dissolved, acidified with 2 mL. of aqua fortis and diluted to 100 mL. with distilled water. In order to receive the homogeneity of solutions, both volumetric flasks with honey solutions were placed into the ultrasonic bath for the next 15 min. The calibration curve for every analysed isotope was composed. The correlation coefficient of calibration data was 0.9995. Using ICP – MS system, the results are presented as mass spectra and relative intensity of metal isotopes and the concentration of metals recalculated into μg/kg. The definitive level of metals in honey samples was obtained by the estimation of the average of metal isotopes. The statistical programme „Minitab" [MINITAB, 1999] was used for processing the research results.

FIGURE 1. Honey sampling points in Lithuania: 1–Stirniai (Labanoras forest), 2–Birstonas, 3–Ukmerge, 4–Kupiskis, 5–Kazlu Ruda, 6–Salantai (Klaipeda region), 7–Elektrenai, 8–Matuizos (Varena region), 9–Joniskis, 10–Silale, 11–Kaunas (Vilijampole, Kleboniskis), 12–Siauliai, 13–Krakes (Kedainiai region).

RESULTS AND DISCUSSION

The valuable characteristics of honey as a natural bioproduct depend on its sort and origin. The quantitative and qualitative relation of chemical elements are characteristic to each blossom of the plant from each region of the country, so general quantity of mineral materials depends on the location. It is possible to determine the origin of the specific samples of honey and the environmental pollution of the region from the quantitative and qualitative relation of heavy and rare metals in honey [Conti & Botre, 2001]. Pb, Cd, Cu and Zn belong to the group of the biogenic elements the concentrations of which in honey is regulated by MTL or standards of microelements in nutrition products [LR HN 54, 2001].

The content of Pb in honey samples is presented in Table 1. The maximum level of this metal – 24.10 μg/kg – was found in the honey collected in Kaunas city territory, however it did not exceed MTL [LR HN 54, 2001].

The presumption can be made that such volume of Pb was found due to the locations of apiaries – the city territory (Vilijampole), suburb (Kleboniskis) or the vicinity between streets of the city and highways, whereas it is obvious that the internal-combustion engines are the main source of contamination with Pb. The minimum amounts of Pb were determined (Figure 2) in the honey collected in the eco-friendlier areas of Lithuania, in the North of the country, which is far away from highways and did not have industrial giants or thermoelectricity stations: in Joniskis (3.20 μg/kg), in Silale and in Salantai, Klaipeda region (4.10 μg/kg). In comparison, weekly standard of Pb per person (weight ~60 kg) in Germany is 1500 μg [Souci *et al*., 1994]. If 10 kg of honey collected in Kaunas city territory which possesses the highest level of Pb, or 30 kg of honey, bearing medial concentrations of this

TABLE 1. Box–Whisker dissemination of HMs concentration (μg/kg) in honey.

	Pb	Cd	Zn	Cu
Min.	3.20	3.90	564.00	110.60
25%	4.10	5.85	1071.00	142.80
Av.	7.50	7.40	1235.00	208.60
75%	11.90	9.90	3205.00	267.80
Max.	24.10	16.50	5008.20	389.40

Av. – mean average value.

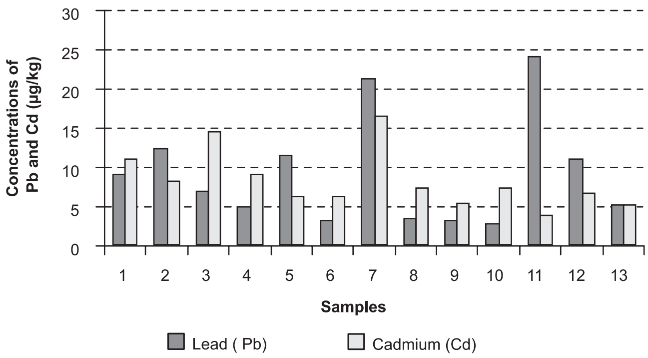

FIGURE 2. Concentrations of lead and cadmium in honey samples.

microelement were eaten, allowable daily standard would be exceeded.

The results of this research were compared with findings of other researchers. It should be noted that the concentrations of Pb in honey established by other investigators were higher; for example in Polish honey – 0.009-0.071 mg/kg [Przybyłowski & Wilczyńska, 2001; Wieczorek *et al*., 2006], in Hungarian – 3.2-186 μg/kg [Caroli *et al*., 2000], and in Turkish – 0.22-103 mg/kg [Sahinler *et al*., 2009]. However, similar concentrations of Pb in honey are presented in the other articles [Yazgan *et al*., 2006; Rahed & Soltan, 2004].

The concentration of Cd in honey varies from the minimum of 3.90 μg/kg to the maximum of 16.50 μg/kg, which was determined in honey collected in Elektrenai region. The average concentration of Cd in Lithuanian honey reaches 7.40 μg/kg, whereas weekly standard of Cd per person (weight ~60 kg) in Germany is 420 μg [Souci *et al.*, 1994]. Therefore, if one consumes 4-14 kg of Lithuanian honey per day, the allowable daily standard would not be exceeded. In the proceedings of other investigators various concentrations of Cd in honey were presented: some similar to the results of this analysis [Garcia *et al*., 2003; Rahed *et al.*, 2004], some found to be lower when the concentration of Cd varied from 0.50 μg/kg to 0.70 μg/kg [Camina *et al*., 2004; Caroli *et al.*, 2000] and some found to be higher – from 12 μg/kg to 63 μg/kg of Cd in honey [Bogdanov *et al.*, 2007; Dobrzański, 1994; Fernandez-Torres *et al.*, 2005].

Cu and Zn are very important microelements that ensure the natural functioning of human organism. They both constitute the structure of proteins and enzymes and take part in the synthesis of proteins and metabolism. The lack of Zn in organism could result in the decrease of immunity system, longer wounds' healing periods, depression, vision, taste and smell disorders, and impotence. However, high levels of these microelements are harmful to a human organism and can result in disorders of cellular metabolism [Eriksson *et al.*, 2004].

The concentration of Zn in honey depends on geographic location of apiary, acidity of the ground and particularly on the instruments used in apiaries, centrifugation and storage of honey, transport utilities and breaches of the technological process. The average concentration of Zn exceeds 1235.00 μg/kg, while the maximum concentration of Zn is 5008.20 μg/kg. According to works of other authors, there are cases when the concentration of Zn in honey varies in larger amounts, differing from 500 μg/kg to 19500 μg/kg [Iskander, 1995].

The concentrations of Cu in honey samples vary from 110.60 μg/kg to 389.40 μg/kg, while the average concentration of this microelement in honey exceeds 208.60 μg/kg. However, there are data from other researchers stating that the concentrations of Cu in honey exceed the one obtained during this research from 2–10 times [Fernandez-Torres *et al.*, 2005] to 5-14 times [Yilmaz *et al.*, 1999].

The levels of Sr in honey samples vary in particular. The minimum and the maximum concentrations of this microelement vary over 10 times, while the average number of Sr reaches 106.50 μg/kg (Table 2).

According to the Rb levels, honey can be assorted into honey of mead and forest. The honey of forest is dark and contains a high quantity of Rb. The maximum level of this microelement was found in honey which was collected in the forest of Labanoras, nearby Stirniai, while the minimum one – in honey collected in town of Siauliai [Gaidamavicius, 2005].

The medium amount of Ba in honey samples exceeds 36.60 μg/kg, though its concentration varies from the minimum of 5.80 μg/kg in honey collected in Krakes (Kedainiai region) to the maximum of 71.30 μg/kg in honey collected in the forest of Labanoras, nearby Stirniai.

As the group of microelements includes rare elements as well, it was very important to determine the levels of lactinoids and actinoids in honey. In the samples of Lithuanian honey examined they were detected only in trace amounts ($1x10^{-9}$ g/kg). The average concentrations of these rare elements are: Ce – 6.50 ng/kg, La – 2.70 ng/kg, and U – 5.60 ng/kg. The vast majority of these elements were found in honey which was collected in the territory of Kaunas city, whereas slightly smaller amounts – in honey collected in Elektrenai. These rare elements were not found in samples of German honey, although the same instruments of analysis were used.

TABLE 2. Box–Whisker dissemination of trace elements concentration (μg/kg) in honey.

	Sr	Rb	Ba	Ce*	La*	U*
Min.	19.50	193.00	5.80	3.90	1.80	0.80
25%	74.40	459.00	16.55	4.50	2.35	3.40
Av.	106.50	625.00	36.60	6.50	2.70	5.60
75%	150.30	755.00	47.70	10.40	3.20	6.80
Max.	240.90	2448.50	71.30	31.90	16.00	8.00

* – concentration in ng/kg; Av. – mean average value.

TABLE 3. The concentration of chlororganic compounds (μg/kg) in honey.

	DDT	DDD	DDE	α-HCH	γ-HCH	PCB
Min.	0.30	0.10	0.10	0.01	0.01	-
Av.	0.41	0.19	0.20	0.09	0.02	-
Max.	0.56	0.33	0.29	0.20	0.04	-

Av. – mean average value.

The investigation was carried out in order to determine the level of residues of organochlorine pesticides – DDT, its metabolites: DDD and DDE also the concentrations of α-HCH and γ-HCH and polychlorinated biphenyls (PCB). The dissemination of COC in honey samples is presented in Table 3.

Polychlorinated biphenyls were not found in the examined samples of Lithuanian honey and only the prints of organochlorine compounds: α-HCH (0.01÷0.20 μg/kg) and γ-HCH (0.01÷0.04 μg/kg), as well as DDT (0.30÷0.56 μg/kg), its metabolites: DDE (0.10÷0.29 μg/kg) and DDD (0.10÷0.33 μg/kg) were observed.

CONCLUSIONS

1. The concentrations of different harmful heavy metals in Lithuanian honey are very broad-based: Pb – 3.20-24.10 μg/kg, Cd – 3.90-16.50 μg/kg, Cu – 110.60-389.40 μg/kg, Zn – 564.30--5008.20 μg/kg, but stay within Maximum Tolerable Limit of Lithuanian Standards of Hygiene. The levels of given microelements in Lithuanian honey are lower than these reported in honey of other EU Countries.

2. According to the Rb levels, honey can be assorted into honey of mead and forest. Honey collected in forest of Labanoras was shown to contain 2248.50 μg/kg of Rb; which is 4 ÷ 12 times higher then in the other samples of honey examined in the study.

3. In the samples of Lithuanian honey very small amounts of α-HCH and γ-HCH (0.01÷0.02 μg/kg and 0.01÷0.04 μg/kg), DDT and its metabolites (0.10÷0.56 μg/kg) were observed. Polychlorinated biphenyls were unrecorded absolutely.

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